APPENDIX 1

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Summary - Operations Involved in the Philip Morris Hybridoma Project.(1990)

Hapten was conjugated to carrier protein at the Philip Morris Research Center. Human serum albumin (HSA) and thyroglobulin were used as carriers. Initially, hapten-HSA was emulsified with complete Freund's adjuvant and was injected IP into female Balb/c mice. The mice received additional doses of the hapten-HSA conjugate at 3 week intervals. For cell fusion, the spleen was asceptically removed from an immunized mouse and the cells were teased into Dulbecco's Modified Eagle's Medium (DMEM). These cells were fused with myeloma cells (NS1) which were grown in culture. Hybridoma formation was initiated by pelleting the spleen and myeloma cells and resuspending them in a polyethylene glycol solution prepared in DMEM by the method of G. Kohler and C. Milstein (Nature 256, 495 (1975), Eur. J. Immunol. 6, 511 (1976)). The cells were transferred to a Hypoxanthine, Aminopterin, Thymidine (HAT) selection medium. In the following days, the spleen cells die naturally, and the unfused myeloma cells are destroyed by the HAT medium. Only hybridomas which contain the genetic material of both the spleen and myeloma cells will grow. Under our accelerated growth conditions, the wells can be scored for colonies by day 5, and they can be assayed by ELISA assay by day 9 post fusion. In order to determine which cells are producing antibodies against the hapten, the second carrier protein (i.e. hapten-thyroglobulin (THG)) was used in the Enzyme-Linked ImmunoSorbant Assay (ELISA). Specifically, hapten-THG was adsorbed to 96 well vinyl assay plates. Supernatants (50-100 µl) from the wells that contained clones were then transferred to the corresponding wells in the assay plates. Following incubation, the wells were washed and a solution of anti-IgG and anti-IgM Goat AntiMouse Antibody (GAMA) conjugated with alkaline phosphatase was added to each well. Following incubation, the wells were washed to remove any unbound GAMA, and a solution of substrate (p-nitrophenyl phosphate) was added. Those wells that contained antihapten antibodies turned yellow due to the antigen initiated accumulation of alkaline phosphatase in the wells. The positive wells were expanded to 2ml and were reassayed by day 14. Positive supernatants were then sent to Philip Morris for further evaluation against other endpoints in order to determine which clones are producing antibodies that would be useful for their specific projects. In some cases, it was necessary to provide larger volumes of supernatant from select clones for a more rigorous evaluation. In other cases, ascities fluid was chosen as the source of antibody. With ascities fluid the yield of antibody can reach 5 -10 mg/ml and can provide a large amount of a good antibody for further study.

It should be noted that Litron Laboratories has also developed proprietary hybridoma technology that has been used in some parts of the project. This methodology permits us to increase the number of activated lymphocytes, and increase the yield of hybridomas following cell fusion. This technology reduces the amount of antigen needed during the immunization phase of the project. Other aspects of our technology reduce the loss of hybridomas during the subcloning stage, where good antibody producers often die. As a consequence, we are able to provide good yields of antigen specific antibody. Select hybridomas from this project have been frozen in liquid nitrogen for permanent storage and can be revived as they are needed.

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